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Description

Nucleic acid hybridization assays are used as a tool for the detection and identification of a target genetic material such as DNA or RNA. Such detection and identification can be for a specific DNA or RNA sequence or specific gene or a point mutation or deletion of a DNA or RNA sequence or gene. A number of techniques exist to carry out such assays. (see Methods In Enzymology, Vol. 68, R. Wu (Ed) pp. 379-469, 1979; and Dunn, A.R., and Sambrook, J., Methods In Enzymology, Viol. 65; Part 1, pp. 468-478, 1980). One of the most widely used procedures is called the Southern blot filter hybridization method (Southern, E., J. Mol. Biol. 98, 503, 1975). This procedure is usually used to identify a particular DNA fragment separated from a mixture of DNA fragments by electrophoresis. The procedure is generally carried out by isolating a sample of DNA from some microorganisms The isolated DNA is subjected to a restriction endonuclease digestion and electrophoresed on a gel (agarose, acrylamide, etc.). When the gel containing the separated DNA fragments is put in contact (blotted with a nitrocellulose filter sheet or diazotized paper, etc.), the fragments are transferred and become bound to the nitrocellulose sheet. The gel-transfer nitrocellulose sheet containing the DNA fragments is then heated to denature the DNA. At this point the sheet is treated with a solution containing a denatured labeled DNA probe and hybridization is allowed to take place. The unhybridized labeled DNA probe is then washed away. The label of the DNA probe is then detected.

It is known to carry out a homogeneous hybridization assay based upon non-radiative energy transfer. This hybridization assay system utilizes a chemiluminescent catalyst and an absorber/emitter moiety. The system involves the use of two polynucleotide reagent strands in such a way that the hybridization assays carried out are in a homogeneous fashion. This means that the target polynucleotide sequence can be detected and identified in solution without the need to carry out any immobilization procedures. The method comprises contacting the target genetic material, under hybridization conditions, with first and second single stranded polynucleotide reagent segments which are complementary to substantially mutually exclusive portions of the target single stranded polynucleotide. The first reagent segment has a chemiluminescent catalyst and the second reagent segment has an absorber/emitter moiety positioned such that, upon hybridization with the target single stranded polynucleotide, the chemiluminescent catalyst and absorber/emitter moiety are close enough in proximity to permit non-radiative energy transfer. The single stranded polynucleotide sample is then contacted with chemiluminescent reagents effective for causing light emission in the presence of the chemiluminescent catalyst. The quantity of light emitted by the absorber/emitter moiety is then measured by an appropriate instrument which thereby indicates the presence of the sample single stranded polynucleotide. This method is disclosed in European Patent Application Publication Number 0 070 685, published January 26, 1983.

This invention provides methods for the detection of a target genetic material having a desired base sequence or gene. Also disclosed are methods for the detection of mutations, such as point mutation or the deletion of a gene or base. Also provided are components for use in such methods.

The methods are based upon techniques which utilize two labeled singled stranded polynucleotide segments which are complementary to the opposite strands of the target genetic material. The methods of the invention result in the formation of a double hybrid and/or a multihybrid, defined hereinbelow.

The method of detection of the double hybrid and the multihybrid is dependent upon the choice of label.

Each single stranded polynucleotide segment can be either part of the same polynucleotide segment i.e., one probe which comprises two polynucleotide segments of interest or can be two separate polynucleotide segments, i.e., two probes with each probe comprising a polynucleotide segment of interest. The label of each probe can be a particle, a moiety which is capable of generating a signal, either directly, e.g., a radioactive label, or indirectly, e.g., an enzyme-linked system or a system wherein each label alone can not create a signal, but when such labels are brought into contact, a signal can be generated.

The invention will be better understood by reference to the attached Figures wherein:

FIGURE 1 represents another embodiment of the invention wherein the two single stranded polynucleotide segments, 70 and 72, are part of the same polynucleotide segment 74. The denatured target genetic material 80 comprises to (+) strand 82 and the (-) strand 84. The (+) strand 82 comprises region 86, region 88 and region 90. The (-) strand 84 comprises region 92, region 94 and region 96. Single stranded polynucleotide segments 70 and 72 are complementary to regions 86 and 96, respectively. When the method of the invention is carried out, numerous single stranded polynucleotide segments 70 and 72 hybridize to regions 86 and 96, respectively, to form the multihybrid 98.

This invention provides methods for the detection of genetic material such as DNA or RNA. The

methods are based upon techniques which utilize two single stranded polynucleotide segments, with each segment comprising a label. Each single stranded polynucleotide segment is complementary to the opposite strands of the target genetic material. When the two single stranded polynucleotide segments are separate segments, there are two polynucleotide probes; when the two single stranded polynucleotide segments are part of the same polynucleotide segment there is one polynucleotide probe, albeit such one polynucleotide probe comprises two single stranded polynucleotide segments of interest. Thus, when the method of the invention is carried out, a double hybrid is formed which comprises two polynucleotide probes joined by their hybridization to the target genetic material (hereinafter referred to as the "double hybrid"). Also, depending upon the choice of label and whether or not the two single stranded polynucleotide segments are separate polynucleotide segments or are part of the same polynucleotide segment, the double hybrid can be interconnected so as to form a multihybrid (hereinafter referred to as the "multihybrid"). Both the double hybrid and the multihybrid are detectable.

The double hybrid or the multihybrid, and therefore, the target genetic material, is detectable by one of three methods. The method that can be utilized to detect the double hybrid or multihybrid is dependent upon what kind of label is utilized. First, the double hybrid or multihybrid is detectable by the fact of the formation of the double hybrid or multihybrid itself. The double hybrid or multihybrid is directly detectable as a precipitate or a glob or a glob-like structure. This precipitate or glob or glob-like structure itself separates from the polynucleotide probes which did not form the double hybrid or multihybrid. This result can be obtained when each label is, for example, a particle. This is an agglutination hybridization assay.

The second method that can be utilized to detect the double hybrid or multihybrid is obtained when one of the labels is, for example, a particle and one of the labels is an entity that is capable of creating a signal, e.g., a radioactive label or an enzyme linked system. The detection of the double hybrid or multihybrid by this method may require a separation step to separate the double hybrid or multihybrid from those polynucleotide probes which comprise such entities which are capable of creating a signal which have not formed the double hybrid or multihybrid. Otherwise, such unhybridized polynucleotide probes may create a signal which can result in a false positive result. This is commonly referred to as "background".

The third method in which the double hybrid or multihybrid is detectable is if each label alone is neither capable of creating a signal itself, either directly or indirectly, nor is a particle, but when the double hybrid or multihybrid is formed, each label of the double hybrid or multihybrid can come into contact and thereby create a signal.

The method of the invention can be carried out with great simplicity. The target genetic material is denatured, i.e., rendered in single stranded form, in an appropriate solvent system by conventional techniques such as by heating or adding a strong base. The target genetic material is then contacted with the polynucleotide probe system under hybridization conditions. But, it should be noted that the polynucleotide probe system can be added to the solvent system either before, during or after the denaturation of the target genetic material. It is preferred to add a vast excess of the polynucleotide probe system. This enhances the likelihood that the double hybrid or multihybrid will be formed. It is essential that the polynucleotide probes be single stranded when they contact the target genetic material. Otherwise, the polynucleotide probes will not be able to hybridize with the target genetic material. However, the polynucleotide probe can be in double stranded form and then denatured and then utilized to contact the target genetic material. This denaturation can be carried out in the solvent system at the same time the target genetic material is being denatured. It should be noted that it is preferred that when the label of the polynucleotide probe is a particle, that the polynucleotide segment be in single stranded form. Otherwise, when the target genetic material is contacted by the polynucleotide probe under hybridizing conditions, the double strands of the polynucleotide probe can easily renature. This can be carried out by, for example, deriving the single stranded polynucleotide segment from a single stranded DNA or RNA phage, separating the (+) and (-) polynucleotide segments and attaching either the (+) or the (-) polynucleotide segment to the particle or attaching both the (+) and (-) polynucleotide segments to the particle in such a fashion that they can not hybridize to each other on the same or different particles.

The double hybrid or multihybrid can now be detected. However, depending on the choice of label and whether or not each polynucleotide probe comprises a single stranded polynucleotide segment which is a separate polynucleotide segment or part of the same polynucleotide segment, those polynuclectide probes which did not form the double hybrid or multihybrid may then have to be separated from those polynucleotide probes which formed the double hybrid or multihybrid.

As disclosed hereinabove, the two single stranded polynucleotide segments can be either two separate polynucleotide segments, i.e., a two

polynucleotide probe system, or they can be part of the same polynucleotide segment, i.e., a one polynucleotide probe system.

The Two Single Stranded Polynucleotide Segments As Two Separate Segments

The two single stranded polynucleotide segments can be two separate polynucleotide segments, with each polynucleotide segment being labeled. In this embodiment of the invention, there are two polynucleotide probes. Each single stranded polynucleotide segment is complementary to substantially mutually exclusive portions of the same or the opposite strands of the target genetic material.

When each single stranded polynucleotide segment is complementary to opposite strands of the target genetic material, it is preferred that each single stranded polynucleotide segment be totally mutually exclusive. This reduces the possibility of each single stranded polynucleotide segment hybridizing to each other when the method the invention is carried out, thus resulting in a background signal. Also, when each single stranded polynuclectide segment is complementary to opposite strands of the target genetic material, it is essential that each single stranded polynucleotide segment be selected such that when each is hybridized to its complementary strand of the target genetic material, there is at least one sequence of both strands of the target genetic material that is available to hybridize to each other. This is due to the fact when the method of the invention is carried out, the opposite strands of the target genetic material will hybridize to each other to form the double hybrid.

As disclosed hereinabove, the double hybrid can be detected by one of three methods, depending upon the choice of the label for each polynucleotide probe.

In a preferred embodiment in the practices of the present invention, each polynucleotide probe is labeled with a particle. When the methods of the invention are carried out with the label of each polynucleotide probe being a particle, the resulting double hybrid comprises two particles which are separable from those polynucleotide probes labeled with a particle which did not form the double hybrid. (Also, as a less preferred embodiment, more than one particle can be attached to each polynucleotide probe). However, it is particularly preferred that each particle comprise numerous single stranded polynucleotide segments. This results in the formation of the multihybrid due to the particles bridging the double hybrids. The multihybrid forms a precipitate or glob or glob-like structure which itself is much more readily detectable than the double hybrid.

The particle can be a macroparticle or a microparticle wherein a microparticle is in solution or preferably a suspension in the solvent system. The particles can be made from a variety of materials including glass, nylon, polymethacrylate, polystyrene, polyvinylchloride, latex, chemically modified plastic, rubber, red blood cells, a polymeric material or biological cells. Such particles may be readily obtained from or manufactured from material obtained from a variety of sources including, for instance, Polysciences, Inc., Pennsylvania.

The single stranded polynucleotide segment can be attached to the particle by any technique. For example, the single stranded polynucleotide segment can be covalently attached to the particle, attached by nonspecific binding or attached by means of the formation of a complex of the particle and the single stranded polynuclectide segment. Complex formation is the noncovalent binding between complementary portions of two molecules. For example, the particle can be coated with avidin and the single stranded polynucleotide segment can be labeled with biotin wherein such segment will then complex with the avidin. Essentially any ligand and receptor can be utilized to complex the polynucleotide segment with the particle. Suitable ligands and receptors include a polynucleotide sequence to be recognized by its complementary sequence, an antibody portion to be recognized by its corresponding antigen, a hormone to be recognized by its receptor, an inhibitor to be recognized by its enzyme, a co-factor portion to be recognized by a co-factor enzyme binding site, a binding ligand to be recognized by its substrate, e.g., biotin-avidin (and any analogs or derivatives thereof) or sugar-lectin, with the biotin-avidin system being preferred. When the single stranded polynucleotide segment is attached to the particle by complex formation, it is preferred to add the particle to the solvent system after the target genetic material is contacted by the single stranded polynucleotide segment.

It is preferred that the single stranded polynucleotide segment be attached to the particle through a moiety. This enhances the ability of the single stranded polynucleotide segment to hybridize with the target genetic material. Suitable moieties are essentially any moiety including, for example, synthetic and natural polymers and oligomers, and preferably polynucleotides. Preferably, each single stranded polynucleotide segment has terminally attached thereto an oligonucleotide tail which is not complementary to the target genetic material. Each single stranded polynucleotide segment can then be attached to the particle through the oligonucleotide tail by any of the techniques described hereinabove, except for the nonspecific

binding.

The second method in which the target genetic material can be detected is wherein one of the pnlynucleotide probes is labeled with a particle and the other polynucleotide probe is labeled with a moiety that is capable of generating a signal.

The particle can be any of those particles described hereinabove.

The moiety that is capable of creating a signal encompasses virtually any of the signal generating systems used in the prior art and any system to be developed in the future. It comprises a moiety which generates a signal itself, e.g., a dye, a radioactive molecule, a chemiluminescnet material, a fluorescent material or a phosphorescent material, or a molety which upon further reaction or manipulation will give rise to a signal, e.g., an enzyme-linked system.

Suitable enzymes that can be utilized to create a signal are essentially any enzyme that is capable of generating a signal when treated with a suitable reagent. Preferred enzymes are horseradish peroxidase, alkaline phosphatase, glucose oxidase, peroxidase, acid phosphatase and β-galactosidase. Such enzymes are preferred because they are very stable, yet highly reactive.

This method is a less preferred embodiment of the invention. This is due to the fact that during the detection step of the method of the invention, it is essential to carry out a procedure to separate the polynucleotide probes which comprise a moiety that is capable of creating a signal that did not form the double hybrid from those polynucleotide probes which did form the double hybrid. This separation can be carried out by sedimentation, centrifugation or filtration or other known techniques.

The third method in which the target genetic material can be detected is wherein each single stranded polynucleotide segment has a label attached thereto wherein when the double hybrid is formed, each label of each single stranded polynucleotide segment has an affinity or is capable of complexing and then is capable of creating a signal. Neither label of each polynucleotide probe alone is capable of creating a signal.

In this system, it is preferred that each of the two labels be attached, either covalently or via complex formation, at one end of each single stranded polynucleotide segment. Thus, it is preferred that the first label be attached in the three prime (3') terminal position of the first single stranded polynucleotide segment and that the second label be attached at the five prime (5') terminal position of the second single stranded polynucleotide segment. Therefore, when the double hybrid is formed, the labels will be positioned as close as possible to each other. However, it is particularly preferred that both strands, i.e., the (+) and (-) strand of each polynucleotide probe, be labeled with the same label. By both strands of the single stranded polynucleotide segment being labeled with the same label, there is no background signal. This is because during the hybridization step of the method of the invention if such polynucleotide probes hybridize to each other, no signal is created.

In this system, it is preferred that the label of each polynucleotide probe have an affinity or be able to form a complex. It is believed that this arrangement assists in increasing the proximity of the two labels. This will result in a stronger signal. Such affinity or complex formation can be naturally occurring, e.g., an apoenzyme is one label and such apoenzyme's cofactor as the other label. In this system, a signal can be created by adding a suitable reagent, but such signal is only created if the apoenzyme and its cofactor form a complex. The affinity or complex can also be artificially created. For example, one label can be a chemiluminescent catalyst and the other label can be an absorber/emitter moiety wherein each of such labels has attached thereto oligonucleotides that are complementary. Such oligonucleotides will hybridize to each other and keep the chemiluminescent catalyst and absorber/emitter moiety in extremely close proximity which will render such system efficacious. This system can be carried out as described in European Patent Application Publication Number 0 070 685, published January 26, 1983, All of the ligand and receptors disclosed hereinabove can be utilized to create the artificial affinity.

In another aspect of the third method of detection of the target genetic material numerous labels can be attached throughout each single stranded polynucleotide segment. It is believed that the attachment of numerous labels to such segments increases the likelihood that when the double hybrids form that the labels of each of such segments will come into contact with each other. Thus, the signal will then be capable of generation. Such labels can be attached to the single stranded polynucleotide segments by methods described in copending, co-assigned European Patent Applications Publication Numbers 0 063 879, published November 3, 1982 and 0 097 373, published January 4, 1984. These European Patent Application Publications are derived from U.S. Patent Application Serial No. 225, 223, filed April 17, 1981 and U.S. Patent Application Serial No. 391, 440, filed June 23, 1982, respectively U.S. Application Serial No. 225,223 issued as U.S. Patent No. 4,711,955 on December 8, 1987. It is preferred to attach such labels via complex formation. This permits one to add the labels after the complex has been formed.

Otherwise, such labels, due to their bulk, may inhibit each polynucleotide probe from hybridizing with the target genetic material. It should be noted that it is essential that when the label is added after the single stranded polynucleotide segments have been permitted to hybridize with the target genetic material, that the complex utilized to attach the label should be different for each polynucleotide probe. This prevents both labels from being attached to the same single stranded polynucleotide segment which would create a background signal.

In yet another aspect of the third method in which the target genetic material can be detected is wherein one of the labeled single stranded polynucleotide segments is fixed to a matrix, such as nitrocellulose, nylon, polystyrene, polyvinylchloride or a transparent or translucent matrix such as glass. This system has the advantage of specificity; only the target genetic material will be fixed to the matrix. Thus, some genetic material that may cause a background signal will not be fixed to the matrix.

In yet another embodiment of this aspect of the present invention, i.e., two single stranded polynucleotide segments as separate segments, there is provided a capture assay.

In this embodiment of the invention the first polynucleotide segment is labeled with a particle, a moiety which is capable of generating a signal, either directly, e.g. a radioactive label, or indirectly, e.g. an enzyme-linked system or a system wherein the label alone cannot create a signal, but when such label is contacted with another label, a signal can be generated. All of such labels as disclosed hereinabove can be utilized.

The second polynucleotide segment is labeled with a different moiety than the first polynucleotide segment or, preferably, is unlabeled. A different moiety in the context of this embodiment of the present invention exists whenever an effective method exists that can discriminate between the two moieties. In addition, the second polynucleotide segment comprises a moiety that is capable of complex formation.

The moiety that is capable of forming a complex is utilized to form a complex with a moiety that is attached to a matrix, such as nitrocellulose, nylon, polystyrene, polyvinylchloride or a transparent or translucent matrix such as glass. Essentially any ligand and receptor can be utilized to make the complex formation.

Suitable ligands and receptors include a oligo or polynucleotide sequence to be recognized by its complementary sequence, an antigen portion to be recognized by its corresponding antibody, a hormone to be recognized by its receptor, an inhibitor to be recognized by its enzyme, a co-factor portion to be recognized by a co-factor enzyme binding site, a binding ligand to be recognized by its substrate, e.g., biotin-avidin (and any analogs or derivatives thereof) or sugar-lectin, with the biotin-avidin system being preferred. When an oligo or polynucleotide sequence is utilized, it is preferred that the oligo or polynucleotide sequence be a homopolymer or oligomer or a repeating copolymer or oligomer. It is believed that the complex formation by a ligand and receptor is of much higher affinity, efficiency and speed than the complex formation of the target genetic material and each single stranded polynucleotide segment.

When the capture assay is carried out, it is believed that the double hybrid or multihybrid is first formed and then is "captured" by the matrix. Thus, when the method of the invention is carried out, those first polynucleotide segments that were not "captured" by the matrix can be separated from those that did, by washing them from the matrix. In a preferred embodiment of this embodiment of the present invention, the target genetic material is contacted with the first and second polynucleotide segments so as to form the double hybrid or multihybrid and then the matrix is contacted with the double hybrid or multihybrid. This assures that the hybrid or multihybrid will be formed before the second polynucleotide segment is captured by the matrix.

The Two Single Stranded Polynucleotide Segments Are Part Of The Same Polynucleotide Segment

The two single stranded polynucleotide segment can be part of the same polynucleotide segment. In this embodiment of the present invention, there is only one polynucleotide probe, but such polynucleotide probe comprises at least two single stranded polynucleotide segments of interest. The single stranded polynucleotide probe can comprise a label, although a label is not essential, but only preferred. Each single stranded polynucleotide segment can be complementary to the opposite strands of the target genetic material.

When each single stranded polynucleotide segment is complementary to opposite strands of the target genetic material, it is preferred that no portion of each segment be complementary. Also, when each single stranded polynucleotide segment is complementary to opposite strands of the target genetic material, it is essential that each single stranded polynucleotide segment be selected such that when each is hybridized to its complementary strand of target genetic material, there is at least one sequence of both strands of the target genetic material that is available to hybridize to each other. This is due to the fact that when the method of the invention is carried out, the opposite strands of the

target genetic material will hybridize to each other to form the double hybrid, which is the only structure that can form.

In this embodiment of the invention, the multihybrid can be formed without attaching more than one single stranded polynucleotide probe to a label. The target genetic material, rather than the label, joins the double hybrids to form the multihybrids. Thus, a precipitate or glob or glob-like structure can be formed without a label. It is preferred that the polynucleotide probe comprise a label. The label renders the multihybrids more easily detectable because the label can decrease the solubility of the polynucleotide probe. As with the embodiment of the invention wherein the two single stranded polynucleotide segments are separate segments, the label of the polynucleotide probe can be a particle. Preferably, many probes are attached to each particle. This results in the formation of an even larger multihybrid. The same particles as described hereinabove can be utilized. Also, the single stranded polynucleotide probe can be attached to the particle by techniques as described hereinabove.

The label can be an entity other than a particle. The label can be any moiety that decreases the solubility of the polynucleotide probe. For example, the label can be a DNA binding protein that binds DNA. This can be carried out by adding such binding proteins before, during or after the target genetic material is contacted with the polynucleotide probe.

The multihybrid can be rendered more easily detectable by utilizing on a portion of the probes a label which is a moiety which is capable of creating a signal. The attachment of such label permits one to amplify the signal generated by the formation of the multihybrid. In this embodiment it is preferred, but not essential, that during the detection step that the polynucleotide probe which comprises a moiety which can create a signal that does not form the multihybrid, to be separated from those which did form the multihybrid. Such separation step can be carried out very simply due to the fact of the very formation of such multihybrid. It is believed that portion of the polynucleotide probes which did not form part of the multihybrid can just be poured off to separate it from the multihybrid. The moieties which can be utilized to create a signal are the same as those described hereinabove.

The target genetic material can be detected by utilizing a portion of the polynucleotide probes labeled with a first label and a portion of the polynucleotide probes labeled with a second label wherein the first and second label are not capable of creating a signal themselves, but when they contact each other they are able to create a signal. It is preferred that about half of the polynucleotide probes be labeled with the first label and about half with the second label. This maximizes the amount of signal that can be created.

The same first and second labels can be utilized as described hereinabove.

INHIBITION ASSAY

The target genetic material can also be detected by utilizing an inhibition hybridization assay based upon the principles of the invention, i.e., the inhibition of the formation of the double hybrid or multihybrid is measured. This can be carried out with the two polynucleotide probe system. However, it is essential that each single stranded polynucleotide segment be selected such that they are complementary to not only the target genetic material, but also complementary to each other. Also, in this embodiment of the invention one of the polynucleotide probes can be fixed to a matrix, such as a nitrocellulose filter, rather than being labeled. Also, it should be noted that in this embodiment of the invention, an inhibition capture assay can be carried out which is based upon the principles of the capture assay. All of the labels described hereinabove can be utilized. Also, such labels can be attached to the single stranded polynucleotide segment by the techniques described hereinabove.

The method can be carried out with great simplicity. The target genetic material is denatured in an appropriate solvent system, i.e., rendered single stranded, by conventional techniques. The target genetic material is then contacted with the polynucleotide probe system and a "blocking" agent under hybridization conditions. The blocking agents are either, but not both, the (+) or the (-) sequences of polynucleotides which are complementary to either the (+) or (-) strand of the target genetic material, but are not complementary to either of the single stranded polynucleotide segments. The blocking agent prevents each strand of the target genetic material from joining the two polynucleotide probes. However, it should be noted that if the target genetic material is single stranded, e.g., mRNA, then no blocking agent is required. The detection of the presence of the double hybrid or multihybrid can now be accomplished. If no double hybrids or multihybrids are formed or fewer double hybrids or multihybrids are formed, then the target genetic material is present. Also, it should be noted that this method is most efficacious when the concentration of the target genetic material is equal to or greater than the concentration of the polynucleotide probe system.

The assay can also be carried out wherein instead of detecting the presence of the target genetic material by the inhibition of the formation of

the double hybrid or multihybrid, the detection of the target genetic material is determined by the hybridization of one of the polynucleotide probes to the target genetic material. For example, the first polynucleotide probe, which need not be labeled, can be fixed to a matrix in an appropriate first solvent system. A vast excess of this polynucleotide probe, as compared to the second polynucleotide probe and target genetic material is utilized. In a second solvent system, the target genetic material is denatured, i.e., rendered into to single stranded to form. The second polynucleotide probe is then utilized to contact the target genetic material under hybridization conditions. (A blocking agent, if necessary, can be utilized herein for the same reasons as described hereinabove) It is essential that the label of this polynucleotide probe be a moiety which itself is capable of creating a signal, e.g., a radioactive label. Suitable moieties are those described hereinabove. The components of the second solvent system, which are still under hybridization conditions, are then transferred to the first solvent system. Thus, under hybridizing conditions, any or all of the second polynucleotide probe which has not hybridized to the target genetic material can hybridize to the first polynucleotide probe, which is fixed to the matrix. Thus, there will remain in the solvent in solution the second polynucleotide probe which is hybridized to the target genetic material. This can be detected by creating a signal with the label of the second nucleotide probe. This assay can also be carried out by means other than utilizing the first polynucleotide probe for separating the second polynucleotide probe which are hybridized to the target genetic material from those that are not. Any suitable means can be used. For example, an enzyme, such as S1 or micrococal nuclease, that destroys single stranded genetic material can be utilized to destroy the second polynucleotide probes that did not hybridize with the target genetic material. Also, an antibody to double or single stranded DNA or RNA can be utilized to do the separation by fixing such antibody to a matrix in the first solvent system. Also, buoyant density centrifugation or hydroxylappatite chromatography can be utilized for such separation.

DETECTION OF MUTATIONS

The method of the invention can also be utilized to detect a genetic mutation such as a point mutation, an inversion and both a large (greater than about 15 nucleotides) and a small (less than about 15 nucleotides) deletion or insertion, and a genetic change leading to an alteration of a restriction enzyme cleavage site, a restriction enzyme polymorphism site. This can be carried out by utilizing the methods of the invention which result in the formation of the double hybrid or multihybrid. However, it is essential that at least one of the single stranded polynucleotide segments comprise a sequence that is substantially complementary, and preferably completely complementary, to the site of the mutation or the site restriction enzyme polymorphism site, whichever is appropriate. It is also preferred that such segment comprise the flanking nucleotides of the appropriate site. After the formation of the double hybrid or the multihybrid, the double hybrid or multihybrid can be contacted with an appropriate restriction enzyme that can cleave at least one position on a double hybrid if the site is present or absent, depending on the choice of polynucleotide probe. Such cleavage results in the breaking of the double hybrid, and therefore, the multihybrid if the multihybrid is present. The breaking of the double hybrid or the multihybrid is detectable.

The choice of the single stranded polynucleotide probe to be utilized is dependent upon whether a restriction enzyme site that identifies the mutant is present or absent in the mutated genetic material. If the restriction enzyme site is present, then it is essential that the single stranded polynucleotide probe contain the restriction enzyme site sequence. If the restriction enzyme site is absent, then it is essential that the single stranded polynucleotide probe contain the sequence for the restriction enzyme site that identifies the mutant, which site is present in the wild type genetic material.

In another aspect of the invention when at least one of the single stranded polynucleotide probes comprises a sequence complementary to the restriction enzyme site sequence that identifies the mutant that is present in the mutant target genetic material rather than the wild type target genetic material, such a probe can comprise many restriction enzyme site sequences which identify the mutant, each of which is complementary to restriction enzyme site sequences which identify the mutant from different mutant-target genetic materials. Thus, one assay can be utilized to detect the existance of at least one of many possible mutations. It is not necessary to carry out a separate assay to determine the existance of each mutation in this case.

Another embodiment of this aspect of the invention is wherein in the polynucleotide probe system of the invention wherein there are two polynucleotide probes, one of the polynucleotide probes is fixed to a matrix, such as a nitrocellulose filter or a transparent or translucent surface. It is not essential that the fixed polynucleotide probe be labeled, but it is essential that the other polynucleotide probe, which preferably contains the re-

striction enzyme site that identifies the mutation, be labeled with a moiety that is capable of creating a signal, such as those described hereinabove. When the method of the invention is carried out, the double hybrid can be formed, which is fixed to the matrix. The portion of the target genetic material that did not form the double hybrid should be separated from those strands which did form the double hybrid, unless the signal is only capable of formation if the double hybrid is formed. The double hybrid is then contacted with an appropriate restriction enzyme which results in breaking the double hybrid, thereby releasing the moiety that is capable of creating a signal. The disappearance of the moiety that is capable of creating a signal from the matrix or the presence of such moiety in the solution of the solvent system can be detected.

A less preferred embodiment of this aspect of the invention is a method which utilizes only one polynucleotide probe. Such probe contains the restriction enzyme site that identifies the mutation and a label which is a moiety that is capable of creating a signal, such as those described hereinabove. The polynucleotide probe is then fixed to a matrix, such as those described hereinabove, such that the portion of the single stranded polynucleotide segment that comprises the mutant target genetic material is between the portion of the single stranded polynucleotide segment which is fixed to the matrix and the portion of the single stranded polynucleotide segment which comprises the moiety that is capable of creating a signal. The polynucleotide probe is then contacted with the target genetic material, which has been rendered single stranded, under hybridization conditions. The strands of the target genetic material that did not hybridize are separated from those which did hybridize, unless the signal is only capable of formation if the hybrid is formed. The resulting hybrid is then contacted with the appropriate restriction enzyme. The moiety that is capable of creating a signal is then released. The disappearance of such moiety from the matrix or the presence of such moiety in the solution of the solvent system can be detected.

Yet another aspect of this invention is a method for the detection of large mutations, i.e., mutations involving deletions or insertions into the nucleotide sequence of greater than about 15 nucleotides. This method utilizes a polynucleotide probe which comprises a polynucleotide sequence that is complementary to the nucleotide sequence of the insertion or the deletion.

In the practice of this aspect of the invention, it is preferred that a positive assay for deletions is observed when the probe does not react with the mutant genetic material and for insertions a positive assay is observed when the probe is shown to react with the mutant genetic material.

The method can be carried out by any conventional hybridization assay, by the methods of this invention or by any hybridization assay to be developed in the future. Utilizing such probe provides an easy method for the detection of large mutations.

EXAMPLE I:

A simple demonstration of the feasibility of detecting a solubilized target genetic material by the formation of the multihybrid was performed using poly rA linked to agarose beads as the probe and poly rU as the soluble target genetic material. In one assay, titration of a suspension of poly rA - agarose with increasing amounts of poly rU was evaluated by microscopic examination of the resulting suspensions. In each suspension the number of agarose beads that was observed to be overlapping or touching another bead were counted. With increasing amounts of poly rU, the number of said complexes increased indicating that poly rU brought poly rA-linked beads into measurably close proximity of one another.

In a separate experiment, poly rA agarose beads were suspended in buffer on microscope slides with (a) no addition, (b) with added poly rA, and (c) with added poly rU.

Because of the low complexity of the nucleic acid sequences, it was immediately observed that the distribution of beads in (c) was different from that in (a) and (b). In (c), the beads floated together as a mass while in (a) and (b) the beads appeared to float freely of each other. This demonstrated that in some circumstances a macroscopic agglomeration of particles can be obtained in such a detection system.

EXAMPLE II:

This example discloses a method for labeling a first single stranded polynucleotide segment that has poly dG at the 5' end with fluorescein and a second single stranded polynucleotide segment that has poly dG at the 3' end with microperoxidase. Also, the fluorescein containing moiety had attached thereto a thymidine trimer and the microperoxidase has attached thereto an adenine trimer.

STEP I: Formation of 5-(3-aminopropyl) deoxyuridine (Propylamino-dU)

Uridine is reacted with mercuric chloride (5. mmol) at pH 5.0. 5-mercurichloride-dU so produced is reacted with acrylonitrile and lithium tetrachloro-palladiate in methanol yielding 5-(2-cyanoethenyl) dU which is reduced by hydrogena-

tion at 3 atmospheres in the presence of 10% palladium on charcoal in methanol.

STEP II: Formation of 5-(N-fluoroscenyl-3-aminopropyl)-dU (fluorescein-dU)

Propylamine-dU is reacted with fluorescein isothiocyanate at pH 8.5. The product is purified by cellulose chromatography.

STEP III: Formation of 3'-benzoyl-fluorescein-dU

Fluorescein-dU is reacted with di-p-dimethoxytrityl chloride in dry pyridine. The 5'-dimethoxytrityl fluorescein dU is purified by silica gel chromatography and acetylated with benzoyl chloride in dry pyridine. 5'-dimethoxytrityl-3'-benzoyl-fluorescein dU is purified by silica gel chromatography. The compound is detritylated by dissolving in methanol: chloroform, 3:7 (v/v) containing 2% benzene sulfonic acid. The product is purified by silica gel chromatography.

Step IV: Formation of $(dA)_3$-(fluorescein-dU)-3'-OH

$(dA)_3$ fully protected for use in phosphotriester oligonucleotide synthesis was deblocked in anhydrous pyridine with triethylamine. Solvent, amine and acrylonitrile are removed by rotary evaporation. The residue is dissolved in dry pyridine along with 3'-benzoyl-fluorescein-dU (.8 mole equivalent) and triisopropylbenzene sulfonyl tetrazole. The crude product is concentrated by evaporation, dissolved in chloroform and washed with bicarbonate. The oligonucleotide is detritylated by treatment with 2% benzene sulfonic acid in 7:3 chloroform: methanol, chromatographed on preparative TLC (silica gel) and deblocked by treatment of 50°C in concentrated ammonia hydroxide. After evaporation of ammonia the product is purified by HPLC reverse phase chromatography.

STEP V: Formation of 5'-OH-$(dA)_3$-(Fluorescein-dU)-$(dG)_n$-3'-OH (5' labeled-oligo dG)

$(dA)_3$-(fluorescein-dU)-3'-OH (1 ug), 1mM dGTP (2.5 ul), terminal transferase (25 u) and 0.01m $CoCl_2$ (5 ul) are incubated in 0.2m potassium cacodylate buffer pH 7.0 containing 1mM mercaptoethanol (final vol. 40 ul) at 37° for 1 hour. The reaction is stopped by heating at 65°C for 5 minutes. The oligo dG product was purified on oligo dC cellulose.

STEP VI: Formation of $(T)_3$-propylamino-dU

Propylamino-dU is blocked with 2-(tert-butoxy carbononyloxyimino)-2-phenylacetonitrile (BOC-ON). This compound is reacted successively with dimethoxy tritylchloride and benzoyl chloride as previously described for the preparation of 3'-benzoyl fluorescein-dU. The trityl group is selectively cleaved by hydrogenolysis at atmospheric pressure in the presence of 5% palladium on charcoal. The N-BOC-3'-acetyl aminopropyl-dU is condensed with protected $(T)_3$-oligonucleotide, deblocked and purified as described for the preparation of $(dA)_3$-fluorescein-dU.

STEP VII: Preparation of oligo-dG-(8-aminohexyladenosine)

Oligo dG (100 ug.) is reacted with 8-aminohexyladenosine-5'-triphosphate in the presence of terminal transferase in cacodylate buffer as previously described. The oligo-dG is isolated by oligo-dC-cellulose chromatography.

STEP VIII: Preparation of microperoxidase coupled to oligo dG (8-aminohexyladenosine)

Oligo dG-(8-aminohexyladenosine) (100 ug.) and microperoxidase (10 mg) is reacted with 1 mg. 1-ethyl-3-(3-dimethylamino propyl) carbodiimide (EDAC) in 100 ul. 0.1M sodium chloride. The reaction is dialyzed and the coupled product isolated by oligo-dC chromatography.

STEP IX: Preparation of microperoxidase coupled to $(T)_3$ and oligo dG

Microperoxidase coupled oligo dG (mixed with oligo dG) is reacted with $(T)_3$-propylamino-dU in the presence of EDAC. The product is purified by successive chromatography on oligo-dC-cellulose and oligo-dA-cellulose.

Claims

1. A method for the detection of target genetic material which comprises:

providing a first polynucleotide probe comprising at least one first label and at least one first single-stranded polynucleotide segment attached to said first label, said first segment being complementary to a first portion of one strand of said target material;

providing a second polynucleotide probe comprising at least one second label and at least one second single-stranded polynucleotide segment attached to said second label, said second segment being complementary to a second portion of the opposite strand of said target material, said first segment being non-complementary to said second segment and said second portion, and said second seg-

ment being non-complementary to said first portion;

rendering said target material single-stranded;

contacting said first probe and said second probe with said single-stranded target material under hybridizing conditions to form a detectable complex comprising a double hybrid or a multihybrid; and

detecting said complex by means of said first label and said second label.

2. The method according to claim 1, characterized in that said first label is a particle, said second label is a moiety that is capable of creating a signal, and said detecting step further comprises separating unhybridized second probes from said complex.

3. The method according to claim 2, characterized in that said moiety that is capable of creating a signal is selected from a dye, a radioactive molecule: a chemiluminescent material, a fluorescent material, and an enzyme.

4. The method according to claim 1 characterized in that said first polynucleotide probe comprises a first particle to which is attached a plurality of first single-stranded polynucleotide segments complementary to a first portion of one strand of said target material; and

said second polynucleotide probe comprises a second particle to which is attached a plurality of second single-stranded polynucleotide segments complementary to a second portion of the opposite strand of said target material.

5. A method for the detection of target genetic material which comprises:

providing a polynucleotide probe comprising a single-stranded polynucleotide segment comprising a first segment portion complementary to a first portion of a strand of said target material and a second segment portion complementary to a second portion of the opposite strand of said target material, wherein when said first and second segment portions are hybridized to said first and second strand portions, at least one single-stranded portion present on said first strand of said target material is capable of hybridizing to at least one complementary single-stranded portion present on said opposite strand of said target material;

rendering said target material single-stranded;

contacting said probe with said single-stranded target material under hybridizing conditions to agglutinate and form thereby a detectable complex comprising a multihybrid or a double hybrid; and

detecting said complex by means of the agglutination that occurs.

6. A method for the detection of a target genetic material which comprises:

providing

(A) a first polynucleotide probe comprising at least one label, and at least one first single-stranded polynucleotide segment attached to said label; and

(B) a second polynucleotide probe comprising at least one second single-stranded polynucleotide segment having attached thereto a first moiety that is capable of forming a complex with a second moiety, wherein said first segment is complementary to a first portion of one strand of said target material, said second segment is complementary to a second portion of the opposite strand of said target material, and said first strand portion is non-complementary to said second portion; and

(C) a matrix which has said second moiety attached thereto;

contacting said target material under hybridizing conditions with said first probe, said second probe, and said matrix to form a detectable complex; and

detecting said complex by means of said label.

7. A method according to claim 6, characterized in that said first probe is hybridized to said target material through said first segment and said second probe is hybridized to said target material through said second segment prior to the complexation of said first moiety and said second moiety.

8. The method according to claim 7, characterized in that said first and second moieties are independently selected from an oligo- or polynucleotide sequence, an antigen, an antibody, a hormone, a receptor, an inhibitor, an enzyme, a co-factor, a co-factor enzyme binding Site, a binding ligand, and a substrate for a binding ligand.

9. The method according to claim 8, characterized in that said binding ligand and said substrate for a binding ligand are selected from biotin, avidin, a sugar and a lectin.

10. The method according to any of claims 1-9, characterized in that said target genetic ma-

terial is selected from the group consisting of double-stranded or partially double-stranded DNA, RNA, oligo- or polynucleotides, gene sequences and proteins.

Patentansprüche

1. Verfahren zum Nachweis eines genetischen Zielmaterials, umfassend:
Bereitstellung einer ersten Polynucleotidsonde, umfassend mindestens einen ersten Marker und mindestens ein erstes einzelsträngiges Polynucleotidsegment, verknüpft mit dem ersten Marker, wobei das erste Segment komplementär zu einem ersten Teil eines Stranges des Zielmaterials ist;
Bereitstellung einer zweiten Polynucleotidsonde, umfassend mindestens einen zweiten Marker und mindestens ein zweites einzelsträngiges Polynucleotidsegment, verknüpft mit dem zweiten Marker, wobei das zweite Segment komplementär zu einem zweiten Teil des Gegenstrangs des Zielmaterials ist, wobei das erste Segment nicht-komplementär zu dem zweiten Segment und dem zweiten Teil ist, und das zweite Segment nicht-komplementär zu dem ersten Teil ist;
Umwandlung des Zielmaterials in einen Einzelstrang;
Inkontaktbringen der ersten Sonde und der zweiten Sonde mit dem einzelsträngigen Zielmaterial unter Hybridisierungsbedingungen, so daß ein nachweisbarer Komplex gebildet wird, der ein Doppelhybrid oder Multihybrid umfaßt; und
Nachweis des Komplexes mit Hilfe des ersten und zweiten Markers.

2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß der erste Marker ein Teilchen ist, daß der zweite Marker eine Einheit ist, die ein Signal erzeugen kann, und daß der Nachweisschritt weiterhin das Abtrennen von unhybridisierten zweiten Sonden vom Komplex umfaßt.

3. Verfahren nach Anspruch 2, dadurch gekennzeichnet, daß die Einheit, die ein Signal erzeugen kann, ausgewählt ist aus einem Farbstoff, einem radioaktiven Molekül, einem lumineszierenden Material, einem fluoreszierenden Material und einem Enzym.

4. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß die erste Polynucleotidsonde ein erstes Teilchen umfaßt, an das eine Vielzahl von ersten einzelsträngigen Polynucleotidsegmenten geknüpft ist, die komplementär zu einem ersten Teil auf einem Strang des Zielmaterials sind; und
daß die zweite Polynucleotidsonde ein zweites Teilchen umfaßt, an das eine Vielzahl von zweiten einzelsträngigen Polynucleotidsegmenten geknüpft ist, die komplementär zu einem zweiten Teil des Gegenstrangs des Zielmaterials sind.

5. Verfahren zum Nachweis eines genetischen Zielmaterials, umfassend:
Bereitstellung einer Polynucleotidsonde, umfassend ein einzelsträngiges Polynucleotidsegment, das einen ersten Segmenteil, der komplementär zu einem ersten Teil eines Stranges des Zielmaterials ist, und einen zweiten Segmentteil, der komplementär zu einem zweiten Teil des Gegenstrangs des Zielmaterials ist, umfaßt, wobei, wenn die ersten und zweiten Segmentteile mit den ersten und zweiten Strangteilen hybridisiert werden, mindestens ein einzelsträngiger Teil, der auf dem ersten Strang des Zielmaterials liegt, fähig ist zur Hybridisierung mit mindestens einem komplementären einzelsträngigen Teil, der auf dem Gegenstrang des Zielmaterials liegt;
Umwandlung des Zielmaterials in einen Einzelstrang;
Inkontaktbringen der Sonde mit dem einzelsträngigen Zielmaterial unter Hybridisierungsbedingungen zur Agglutination und Bildung eines nachweisbaren Komplexes, der ein Multihybrid oder ein Doppelhybrid umfaßt; und
Nachweis des Komplexes mit Hilfe der auftretenden Agglutination.

6. Verfahren zum Nachweis eines genetischen Zielmaterials, umfassend:
Bereitstellung
(A) einer ersten Polynucleotidsonde, umfassend mindestens einen Marker und mindestens ein erstes einzelsträngiges Polynucleotidsegment, verknüpft mit dem Marker; und
(B) einer zweiten Polynucleotidsonde, umfassend mindestens ein zweites einzelsträngiges Polynucleotidsegment, an das eine erste Einheit geknüpft ist, die zur Bildung eines Komplexes mit einer zweiten Einheit befähigt ist, wobei das erste Segment komplementär zu einem ersten Teil auf einem Strang des Zielmaterials ist, das zweite Segment komplementär zu einem zweiten Teil des Gegenstrangs des Zielmaterials ist, und der erste Strangteil nicht-komplementär zum zweiten Teil ist; und
(C) einer Matrix, an die die zweite Einheit geknüpft ist;
Inkontaktbringen des Zielmaterials unter Hybri-

disierungsbedingungen mit der ersten Sonde, der zweiten Sonde und der Matrix zur Bildung eines nachweisbaren Komplexes; und
Nachweis des Komplexes mit Hilfe des Markers.

7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß vor der Komplexbildung der ersten Einheit und der zweiten Einheit die erste Sonde mit dem Zielmaterial über das erste Segment hybridisiert wird und die zweite Sonde mit dem Zielmaterial über das zweite Segment hybridisiert wird.

8. Verfahren nach Anspruch 7, dadurch gekennzeichnet, daß die ersten und zweiten Einheiten unabhängig voneinander ausgewählt sind aus Oligo- oder Polynucleotidsequenzen, einem Antigen, einem Antikörper, einem Hormon, einem Rezeptor, einem Inhibitor, einem Enzym, einem Cofaktor, einer Cofaktor-Enzym-Bindungsstelle, einem Bindungsliganden und einem Substrat für einen Bindungsliganden.

9. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß der Bindungsligand und das Substrat für einen Bindungsliganden ausgewählt sind aus Biotin, Avidin, einem Zucker und einem Lectin.

10. Verfahren nach einem der Ansprüche 1 bis 9, dadurch gekennzeichnet, daß das genetische Zielmaterial ausgewählt ist aus doppelsträngiger oder teilweise doppelsträngiger DNA, RNA, Oligo- oder Polynucleotiden, Gensequenzen und Proteinen.

Revendications

1. Procédé pour la détection d'un matériel génétique cible, qui comprend les étapes consistant à:
   - fournir une première sonde polynucléotidique comprenant au moins un premier marqueur et au moins un premier segment polynucléotidique monocaténaire lié audit premier marqueur, ledit premier segment étant complémentaire d'une première portion d'un brin dudit matériel cible;
   - fournir une seconde sonde polynucléotidique comprenant au moins un second marqueur et au moins un second segment polynucléotidique monocaténaire lié audit second marqueur, ledit second segment étant complémentaire d'une seconde portion du brin opposé dudit matérial cible, ledit premier segment étant non complémentaire dudit second segment et de ladite seconde portion, et ledit second segment étant non complémentaire de ladite première portion;
   - rendre monocaténaire ledit matériel cible;
   - mettre en contact ladite première sonde et ladite seconde sonde avec ledit matériel cible monocaténaire, dans des conditions d'hybridation, pour former un complexe détectable comprenant un double hybride ou un multihybride; et
   - détecter ledit complexe au moyen dudit premier marqueur et dudit second marqueur.

2. Procédé selon la revendication 1, caractérisé en ce que ledit premier marqueur est une particule, ledit second marqueur est un fragment qui est capable de créer un signal, et ladite étape de détection comprend en outre la séparation des secondes sondes non hybridées d'avec ledit complexe.

3. Procédé selon la revendication 2, caractérisé en ce que ledit fragment qui est capable de créer un signal est choisi parmi un colorant, une molécule radioactive, une substance chimioluminescence, une substance fluorescente et une enzyme.

4. Procédé selon la revendication 1, caractérisé en ce que ladite première sonde polynucléotidique comprend une première particule à laquelle est liée une pluralité de premiers segments polynucléotidiques monocaténaires complémentaires d'une première portion d'un brin dudit matériel cible; et
ladite seconde sonde polynucléotidique comprend une seconde particule à laquelle est liée une pluralité de seconds segments polynucléotidiques monocaténaires complémentaires d'une seconde portion du brin opposé dudit matériel cible.

5. Procédé pour la détection d'un matériel génétique cible, qui comprend les étapes consistant à:
   - fournir une sonde polynucléotidique comprenant un segment polynucléotidique monocaténaire comprenant une première portion de segment complémentaire d'une première portion d'un brin dudit matériel cible, et une seconde portion de segment complémentaire d'une seconde portion du brin opposé dudit matériel cible, lorsque lesdites première et seconde portions de segment sont hybridées avec lesdites première et seconde portions de

brin, au moins une portion monocaténaire présente sur ledit premier brin dudit matériel cible étant capable de s'hybrider avec au moins une portion monocaténaire complémentaire présente sur ledit brin opposé dudit matériel cible;
- rendre monocaténaire ledit matériel cible;
- mettre ladite sonde en contact avec ledit matériel cible monocaténaire, dans des conditions d'hybridation, pour l'agglutiner et former ainsi un complexe détectable comprenant un multihybride ou un double hybride; et
- détecter ledit complexe au moyen de l'agglutination qui se produit.

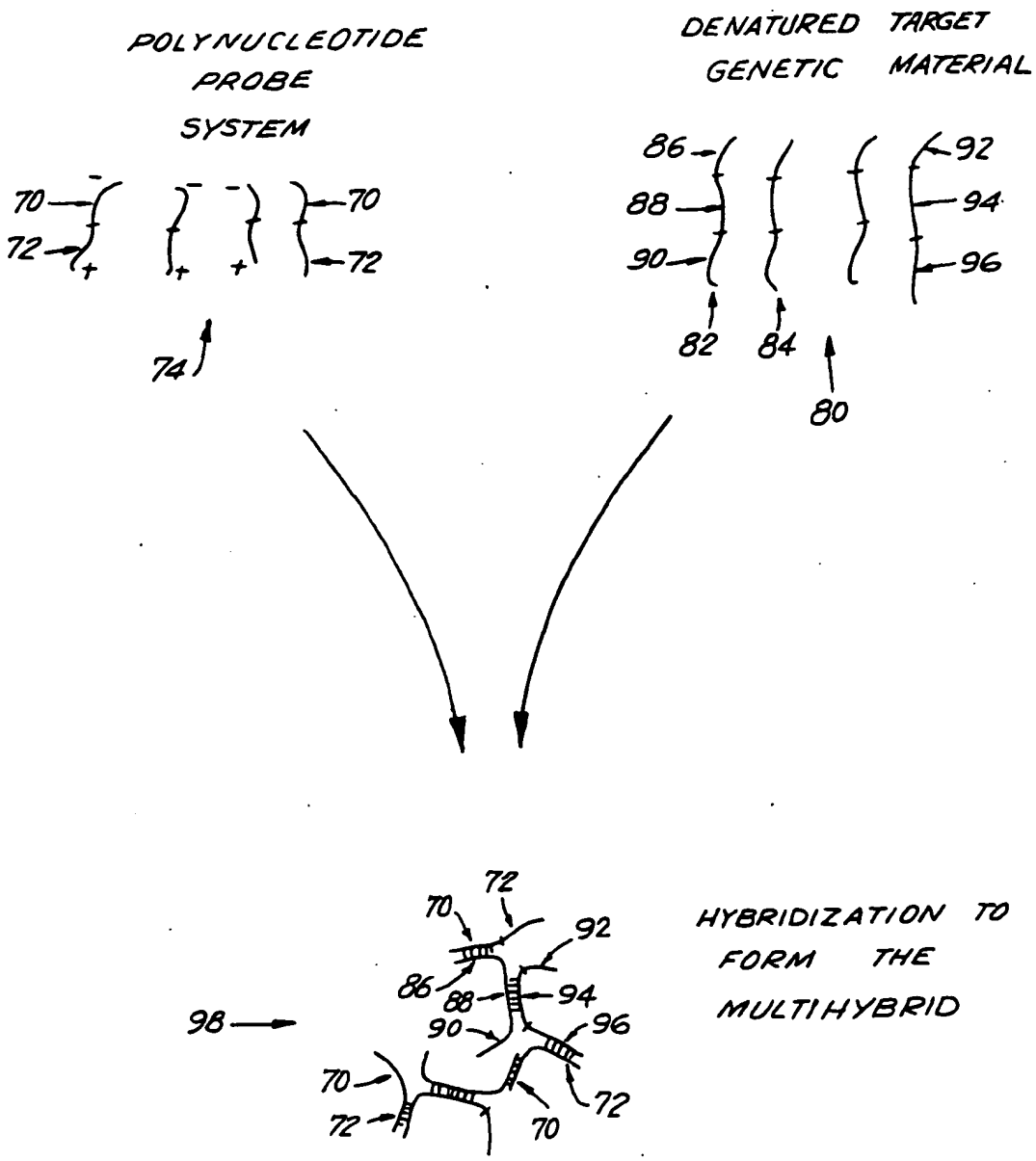
6. Procédé pour la détection d'un matériel génétique cible, qui comprend les étapes consistant à:
   - fournir:
     (A) une première sonde polynucléotidique comprenant au moins un marqueur et au moins un premier segment polynucléotidique monocaténaire lié audit marqueur; et
     (B) une seconde sonde polynucléotidique comprenant au moins un second segment polynucléotidique monocaténaire auquel est lié un premier fragment qui est capable de former un complexe avec un second fragment, ledit premier segment étant complémentaire d'une première portion d'un brin dudit matériel cible, ledit second segment étant complémentaire d'une seconde portion du brin opposé dudit matériel cible, et ladite première portion de brin étant non complémentaire de ladite seconde portion; et
     (C) une matrice à laquelle est lié ledit second fragment;
   - mettre en contact ledit matériel cible, dans des conditions d'hybridation, avec ladite première sonde, ladite seconde sonde et ladite matrice, pour former un complexe détectable; et
   - détecter ledit complexe au moyen dudit marqueur.

7. Procédé selon la revendication 6, caractérisé en ce que ladite première sonde est hybridée avec ledit matériel cible par l'intermédiaire dudit premier segment, et ladite seconde sonde est hybridée avec ledit matériel cible par l'intermédiaire dudit second segment, avant la complexation dudit premier fragment et dudit second fragment.

8. Procédé selon la revendication 7, caractérisé en ce que lesdits premier et second fragments sont indépendamment choisis parmi une séquence oligo- ou polynucléotidique, un antigène, un anticorps, une hormone, un récepteur, un inhibiteur, une enzyme, un cofacteur, un site de liaison d'une enzyme à un cofacteur, un ligand de liaison et un substrat pour un ligand de liaison.

9. Procédé selon la revendication 8, caractérisé en ce que ledit ligand de liaison et ledit substrat pour un ligand de liaison sont choisis parmi la biotine, l'avidine, un sucre et une lectine.

10. Procédé selon l'une quelconque des revendications 1 à 9, caractérisé en ce que ledit matériel génétique cible est choisi parmi un ADN bicaténaire ou partiellement bicaténaire, un ARN, des oligo- ou polynucléotides, des séquences de gènes et des protéines.


POLYNUCLEOTIDE PROBE SYSTEM
DENATURED TARGET GENETIC MATERIAL
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HYBRIDIZATION TO FORM THE MULTIHYBRID

FIG. 1